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EXPERIMENTS DURING 1915 IN THE DESTRUCTION OF FLY LARVÆ IN HORSE MANURE.[1]

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INTRODUCTION.

This bulletin is a report of experiments on the chemical treatment of manure during 1915, conducted with a view to destroying the fly larvæ therein without injuring the manure for agricultural purposes. As in the experiments previously reported, the cooperative arrangement between the Bureaus of Entomology and Chemistry was continued.

The experiments carried out during 1913 and 1914 have been fully reported in Department Bulletins 118 (1)[2] and 245 (2) and include tests of the larvicidal action of a large number of inorganic and organic compounds. Material from several plant sources was included among the organic compounds tested. The results of the experiments during 1913 and 1914 pointed to a number of substances having pronounced larvicidal value, but the majority of these substances did not meet the full requirements, viz, (1) cheapness; (2) noninjurious action on the bacterial flora, on the chemical composition of the manure, and on the growth of plants;[3] (3) lack of extremely toxic properties.

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[1] Experimental work during the summer was conducted on the farm of the Bureau of Animal Industry at Bethesda, Md., and during the autumn at the Louisiana Experiment Station at Baton Rouge, La. The entomological work at Baton Rouge was done by Mr. J. L. Webb, of the Bureau of Entomology; the bacteriological counts at Washington were made by Dr. L. P. Shippen of the Bureau of Chemistry, and at Baton Rouge by Dr. C. W. Edgerton and Dr. Harry Morris of the State Experiment Station. Thanks are due Dr. W. R. Dodson, Director of the Louisiana State Experiment Station, for facilitating the work at Baton Rouge.

[2] Numbers in parenthesis refer to "Literature cited," p. 19.

[3] A detailed discussion of the effect of boron on plants and plant growth is embodied in an article in the Journal of Agricultural Research, vol. 5, No. 19, page 877, "Boron: Its absorption and distribution in plants and its effect on growth," by F. C. Cook.

Of all the substances which were found to be effective, borax was the cheapest, most generally available, and the most easily transported and handled, but it was open to the objection that excessive amounts added to manure (the probable result of carelessness in application) would result in injury to plants grown in soil to which this manure would be applied (3). It was accordingly considered advisable in these experiments to restrict the use of borax to garbage heaps, outhouses, offal of various kinds, in fact, to any possible breeding material not intended for use as a fertilizer, and to recommend powdered hellebore for the treatment of all material likely to be used for fertilizing purposes.

Powdered hellebore, when used at the rate of one-half pound per 8 bushels of manure, showed a larvicidal action about equal to that of borax when the latter was applied at the rate of two-thirds of a pound per 8 bushels, and it was evident that no injurious action either on manure or on the growth of plants followed the application; in fact, the hellebore was found to have disappeared from manure after a period of 30 days. The price of powdered hellebore, with that of drugs in general, has increased considerably during the past 2 years.

The fact that a plant material was found possessing such high larvicidal powers led to an investigation during the past season of various plant materials which were known to contain alkaloids or glucosids, or were thought to possess some value as larvicides. Included in the list to be studied were a number of common weeds, parts of trees, shrubs, etc., which at the present time have no economic value. There seemed to be a possibility that this line of experiments might point to some widely distributed and abundant plant containing a substance specifically poisonous to coprophagous larvæ.

The season's work also included tests with fertilizer mixtures, such as calcium cyanamid and acid phosphate, calcium cyanamid and kainit, etc., with the idea that some combination might be found which would possess a high larvicidal power and also increase the fertilizing value of the manure.

METHODS.

The methods employed were the same in most details as those described in Department Bulletins 118 (1) and 245 (2). The same cages that had been employed during the season of 1914 were found to be satisfactory. As in the previous tests, 8 bushels of fresh larvæ-infested manure were added to each cage and treated in three layers. When any substance was used dry it was also applied in three layers, and each layer of the treated manure was sprinkled with water. The methods for the open-pile experiments were slightly modified, so that instead of collecting and counting the puparia after a period of 6 to 8 days following the treatment, a pyramidal cage having a flytrap at the top was placed over the pile immediately after the last treatment, and all emerging flies collected in the trap were chloroformed and counted. The figures given in the tables are for the house fly only. *Stomoxys calcitrans*, certain species of Phorbia, and various Sarcophagidæ were often present, but in comparatively small numbers. In addition to the cage and open-pile experiments a few tests were made, placing manure in large wooden boxes which were lined with heavy paper to prevent the migration of larvæ through the cracks or corners. Netting and flytraps were attached to the tops of the boxes. Methods of chemical and bacteriological examination of the manure were practically the same as those employed in previous investigations, and described in detail in Department Bulletin 118 (1), pages 3 to 8, with the exception that nitrates were determined colorimetrically by means of diphenylamin. The examinations were made only in the case of a few substances where favorable larvicidal action was shown. The nitrogen results[1] obtained by the distillation method with magnesium oxid (A. O. A. C. method) (7), are included in the tables under the head of amid nitrogen, the ammonia nitrogen as determined by the Folin and Macallum aeration method (4) being deducted from the nitrogen results by the distillation method.

GENERAL ACCOUNT OF SUBSTANCES USED.

Most of the substances tested during the past season fall naturally into two groups: (1) Infusions of poisonous plant materials, and (2) fertilizing mixtures.

PLANT INFUSIONS.

Plant infusions were prepared by mixing a weighed amount of powdered or finely divided material with a measured quantity of water, stirring well, and allowing the mixture to stand overnight. The resulting infusion was used within 24 hours, applying it to the manure with a sprinkling can from which the nozzle was removed if there was any tendency to clog. In this way, the solid as well as the liquid portions of the plant material were directly applied to the manure. The results of cage experiments with plant materials are shown in Table 1, and the results of the open-pile experiments in Table 2.

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[1] All nitrogen determinations were made by the Nitrogen Laboratory of the Bureau of Chemistry.

TABLE 1.—*Destruction of fly larvæ in horse manure—Results with poisonous plant materials.*

[Cage experiments, Bethesda, Md., 1915.]

No.	Material used.	Form used.	Treatment of 8 bushels of manure, using 10 gallons of infusion (amount of plant material used per 10 gallons).	Flies emerged (number).	Apparent larvicidal effect (per cent of control average).	Flies emerged from control cages.	
						Number.	Average.
1	Asclepias (*Asclepias tuberosa*).	Powdered root	A, 1 pound	338	54	385	733
			B, 1 pound	431	42	1,345	
2	Berberis (*Berberis aquifolium*).	do	A, 1 pound	207	60	982	508
			B, 1 pound	40	92	35	
3	Bitter-sweet (*Solanum dulcamara*).	Powdered entire plant.	A, 1 pound	378	26	982	508
			B, 1 pound	92	82	35	
4	Black hellebore (*Helleborus niger*).	Powdered root	A, ½ pound	0	100	385	733
			B, ½ pound	583	21	1,345	
5	Black locust (*Robinia pseudacacia*).	Powdered bark	A, 1 pound	827	50	1,164	1,639
			B, ½ pound	1,009	39	2,114	
6	Castor bean (*Ricinus communis*).	Ground cake	A, 4 pounds	2,317	0	1,141	1,488
			B, 2 pounds	2,433	0	2,161	
			C, 1 pound	2,726	0	1,164	
			D, 1 pound	587	0	982	508
			E, 1 pound	198	62	35	
7	Cinchona (*Cinchona succirubra*).	Powdered bark	A, 1 pound	23	97	385	733
			B, 1 pound	187	75	1,345	
8	Hellebore (*Veratrum viride*).	Powdered root	A, ½ pound[1]	116	92	1,075	1,474
			B, ½ pound[1]	53	97	1,872	
9	Hemlock, poison (*Conium maculatum*).	Powdered fruit	A, 1 pound	976	41	1,164	1,639
			B, ½ pound	1,828	0	....	
			C, ¼ pound	1,492	9	2,114	
10	Jaborandi (*Pilocarpus jaborandi*).	Powdered leaves	A, 1 pound	305	59	385	733
			B, 1 pound	604	18	1,345	
11	Jamaica dogwood (*Piscidia erythrina*).	Powdered bark of roots.	A, 4 pounds	1,045	30	....	1,474
			B, 2 pounds	797	46	1,075	
			C, 2 pounds	672	55	1,872	
			A, 1.35 pounds	573	65	1,164	1,639
			B, 0.67 pound	716	57	2,114	
12	Larkspur (*Delphinium* sp.).	Ground seeds	A, 1 pound	284	81	1,075	1,474
			B, 1 pound	418	72	1,872	
13	Laurel (*Kalmia latifolia*).	Dried leaves	A, 1 pound	3,553	0	1,164 2,114	1,639
			B, ½ pound	2,423	0		
			C, ¼ pound	1,583	0		
14	Quassia (*Picrasma excelsa*).	Powdered chips	A, 4 pounds	44	97	1,141	1,488
			B, 2 pounds	2,501	0	2,161	
			C, 1 pound	727	52	1,164	
15	Soapweed (*Agave lecheguilla*).	Macerated roots	A, 2½ pounds	2,282	0	1,141	1,488
			B, 1¾ pounds	1,308	12	2,161	
			C, ¾ pound	1,123	25	1,114	

[1] Applied dry, 10 gallons water added.

TABLE 2.—*Destruction of fly larvæ in horse manure—Results with poisonous plant materials.*

[Open-pile experiments, Bethesda, Md., 1915.]

No.	Material used.	Form used.	Treatment of 4 bushels manure, using 5 gallons infusion (amount of plant material used per 5 gallons).	Flies emerged (number).	Apparent larvicidal effect (per cent of control average).	Flies emerged from control cages.	
						Number.	Average.
1	Absinthe (*Artemisia absinthium*).	Powdered leaves	A, ½ pound	450	0	391	342
			B, ½ pound	683	0	293	
2	Anthemis (*Anthemis nobilis*).	Powdered flowering heads.	A, ½ pound	331	3.0	391	342
			B, ½ pound	653	0	293	
3	Hellebore (*Veratrum viride*).	Powdered roots	A, ¼ pound	353	93.0	7,192	4,791
			B, ¼ pound	636	87.0	2,391	
			C, ¼ pound[1]	241	51.0	528	492
			D, ¼ pound[1]	96	80.5	456	
4	Poke root (*Phytolacca decandra*).	do	A, ½ pound	1,873	0	528	492
			B, ½ pound	710	0	456	

[1] Applied dry, 5 gallons water added.

The results obtained during 1914 with corn cockle (*Agrostemma githago*), a commercial extract of tobacco (*Nicotiana tabacum*) containing 40 per cent nicotine sulphate, and stramonium (*Datura stramonium*) showed no marked or consistent larvicidal action, and these substances were not tested during the past season.

A few experiments with hellebore (*Veratrum viride, Veratrum album*) were carried out this year to supplement the results of the previous season. The figures obtained both in the cage and open-pile experiments confirm the statements previously made as to its effectiveness. The results in Table 2 indicate that the hellebore infusion which had been standing for 20 to 24 hours was more effective than the hellebore applied as a powder and immediately sprinkled with water.

Two tests were made during the past season, using a 1 per cent infusion of ground larkspur seeds (*Delphinium* sp.). An apparent larvicidal action of 72 and 81 per cent was obtained. In view of the cost and the comparatively low efficiency of this material it is hardly to be considered a practical larvicide.

An infusion prepared from the macerated roots of soapweed (*Agave lecheguilla*) was again tested, but the results were practically negative.

During 1915, 18 plant substances were tested, and a study of Tables 1 and 2 shows that Berberis, Cinchona, and quassia were the only substances which gave indications of having any effect on fly larvæ. Berberis and Cinchona are both expensive, and as apparently large amounts of quassia are necessary to act as an effective larvicide none of these three substances can be considered practicable. Black hellebore (*Helleborus niger*) was used in two experiments, but the results were inconsistent. This plant is not only of a different genus but belongs to a different family of plants than *Veratrum viride* and *Veratrum album.*

FERTILIZING MIXTURES.

During the season of 1913 several tests were made with calcium cyanamid, kainit, and acid phosphate, using each separately. The results were such that no one of these substances could be considered a good larvicide when used in reasonable amounts. As calcium cyanamid and kainit showed some larvicidal action, and as the value of using a fertilizer as a larvicide is obvious, experiments were started during 1915 with the hope of finding an effective fertilizer mixture. In addition to the foregoing, gypsum, Chile saltpeter, and muriate of potash were tried in mixtures. The results of these tests are summarized in Tables 3, 4, 5, and 6.

CHILE SALTPETER ($NaNO_3$) AND ACID PHOSPHATE $CaH_4(PO_4)_2$, $Ca_2H_2(PO_4)_2$, $Ca_3(PO_4)_2$.

A mixture of 2 pounds each of Chile saltpeter and acid phosphate was used in duplicate cage experiments (Table 3, No. 3). The results were poor, showing on the average about a 36 per cent larvicidal action.

TABLE 3.—*Destruction of fly larvæ in horse manure—Results with fertilizer mixtures.*

[Cage experiments, Bethesda, Md., 1915.]

No.	Material used.	Treatment of 8 bushels manure with following amounts of mixtures, then sprinkled with 10 gallons of water.	Flies emerged (number).	Apparent larvicidal effect (per cent of control average).	Flies emerged from control cages.	
					Number.	Average.
1	Calcium cyanamid+ acid phosphate.	A, 1 pound calcium cyanamid+4 pounds acid phosphate.	156	90.5	....	....
		B, 2 pounds calcium cyanamid+ 2 pounds acid phosphate.	16	99.0	1,075	1,474
		C, 1¼ pounds calcium cyanamid+ 2½ pounds acid phosphate.	89	94.0	1,872	
2	.....do...............	A, ¾ pound calcium cyanamid+ 6 pounds acid phosphate.	905	67.5	2,910	2,787
		B, ¾ pound calcium cyanamid+ 6 pounds acid phosphate.	831	70.2	2,665	
3	Chile saltpeter+ acid phosphate.	A, 2 pounds saltpeter+2 pounds acid phosphate.	1.934	30.6	2,910	2,787
		B, 2 pounds saltpeter+ 2 pounds acid phosphate.	1,661	42.5	2,665	
4	Calcium cyanamid+ gypsum.	A, 1 pound calcium cyanamid+ 2 pounds gypsum.	610	78.2	2,910	2,787
		B, 1 pound calcium cyanamid+2 pounds gypsum.	385	86.3	2,665	
5	Borax + gypsum....	A, ¾ pound borax+0.3 pound gypsum..	37	98.7	2,910	2,787
		B, ¾ pound borax+0.3 pound gypsum..	22	99.0	2,665	

CALCIUM CYANAMID ($CaCN_2$) and GYPSUM ($CaSO_4$).

A mixture of 1 pound of calcium cyanamid and 2 pounds of gypsum was used in duplicate in cage experiments at Bethesda, Md., with an average larvicidal action of 82 per cent (Table 3, No. 4). This is very nearly as high as the average larvicidal action for mixtures of 1 pound of calcium cyanamid with varying amounts of acid phosphate, and indicates that gypsum may be used in place of acid phosphate in mixtures with calcium cyanamid.

BORAX ($Na_2B_4O_7$) and GYPSUM ($CaSO_4$).

Although borax is not a fertilizer, it was tried in a mixture with gypsum with the idea that the SO_4 radical in gypsum might tend to reduce the toxic action on plant growth, which is known to result from the application of large amounts of borax. The figures given in Table 3, No. 5, show that borax gave excellent larvicidal results in the presence of gypsum, but the effect of manure treated with this mixture on plant growth was not determined.

CALCIUM CYANAMID, KAINIT (KCl and $MgSO_4$), and ACID PHOSPHATE.

This is an ideal mixture from the point of view of a fertilizer, as it furnishes the three essential elements of plant food, namely, nitrogen, potash, and phosphoric acid.

The results of 8 open-pile experiments, using from one-eighth pound to 1 pound of calcium cyanamid in the mixtures, are recorded in Table 4, Nos. 2 and 3. The larvicidal results were all low and irregular, varying from 10 to 81 per cent.

TABLE 4.—*Destruction of fly larvæ in horse manure—Results with fertilizer mixtures and solutions of calcium cyanamid.*

[Open-pile experiments, Bethesda, Md., and Baton Rouge, La., 1915.]

No.	Material used.	Treatment of 4 bushels of manure with following amounts of mixtures, then sprinkled with 5 gallons of water.	Flies emerged (number).	Apparent larvicidal effect (per cent of control average).	Flies emerged from control cages.	
					Number.	Average.
1	Calcium cyanamid + kainit.	A, 1 pound calcium cyanamid + 1 pound kainit.	93	81.1	377	490
		B, 1 pound calcium cyanamid + 1 pound kainit.	247	49.6	604	
2	Calcium cyanamid + kainit + acid phosphate.	A, ⅛ pound calcium cyanamid + ¼ pound kainit + ½ pound acid phosphate.	1,790	66	7,250	5,392
		B, ⅛ pound calcium cyanamid + ¼ pound kainit + ½ pound acid phosphate.	1,015	81	3,535	
3	....do....	A, 1 pound calcium cyanamid + 1 pound kainit + 4 pounds acid phosphate.	111	33	....	....
		B, 1 pound calcium cyanamid + 1 pound kainit + 4 pounds acid phosphate.	153	10	....	....
		C, 1 pound calcium cyanamid + 2 pounds kainit + 3 pounds acid phosphate.	86	48	....	....
		D, 1 pound calcium cyanamid + 2 pounds kainit + 3 pounds acid phosphate.	50	60	....	....
		E, 1 pound calcium cyanamid + 3 pounds kainit + 2 pounds acid phosphate.	48	61	263	163
		F, 1 pound calcium cyanamid + 3 pounds kainit + 2 pounds acid phosphate.	118	32	62	
4	Calcium cyanamid + muriate of potash + acid phosphate.	A, 1 pound calcium cyanamid + 1 pound muriate of potash + 3 pounds acid phosphate.	1,056	0	378	452
		B, 1 pound calcium cyanamid + 1 pound muriate of potash + 3 pounds acid phosphate.	717	0	526	
5	Calcium cyanamid + acid phosphate.	A, ⅓ pound calcium cyanamid + 2⅔ pounds acid phosphate.	176	19	....	....
		B, ⅓ pound calcium cyanamid + 2⅔ pounds acid phosphate.	221	0	....	....
		C, ⅓ pound calcium cyanamid + 2⅔ pounds acid phosphate.	286	0	238	216
		D, ⅓ pound calcium cyanamid + 2⅔ pounds acid phosphate.	186	14	194	
6	....do....	A, ½ pound calcium cyanamid + ¾ pound acid phosphate.	850 ca.	30	1,123	1,220
		B, ½ pound calcium cyanamid + ¾ pound acid phosphate.	934 ca.	24	1,317	
7	....do....	A, ½ pound calcium cyanamid + 2 pounds acid phosphate.	1,984	63	7,250	5,392
		B, ½ pound calcium cyanamid + 2 pounds acid phosphate.	2,115	60	3,535	
8	....do....	A, ½ pound calcium cyanamid + 3 pounds acid phosphate.	789 ca.	35	1,123	1,220
		B, ½ pound calcium cyanamid + 3 pounds acid phosphate.	1,052 ca.	14	1,317	
9	....do....	A, ½ pound calcium cyanamid + 3 pounds acid phosphate.	1,426	24	2,576	1,868
		B, ½ pound calcium cyanamid + 3 pounds acid phosphate.	1,233	34	1,160	
10	....do....	A, ⅔ pound calcium cyanamid + 1½ pounds acid phosphate.	1,136	13	1,716	1,300
		B, ⅔ pound calcium cyanamid + 1½ pounds acid phosphate.	611	53	884	
11	....do....	A, ¾ pound calcium cyanamid + 1½ pounds acid phosphate.	1,127	40	2,576	1,868
		B, ¾ pound calcium cyanamid + 1½ pounds acid phosphate.	922	50	1,160	
12	....do....	A, ¾ pound calcium cyanamid + 1½ pounds acid phosphate.	1,297	0	1,716	1,300
		B, ¾ pound calcium cyanamid + 1½ pounds acid phosphate.	1,248	4	884	

TABLE 4.—*Destruction of fly larvæ in horse manure—Results with fertilizer mixtures and solutions of calcium cyanamid*—Continued.

No.	Material used.	Treatment of 4 bushels of manure with following amounts of mixtures, then sprinkled with 5 gallons of water.	Flies emerged (number).	Apparent larvicidal effect (per cent of control average).	Flies emerged from control cages.	
					Number.	Average.
13	Calcium cyanamid + acid phosphate.	A, ¾ pound calcium cyanamid + 4 pounds acid phosphate.	2,377	0	....	....
		B, ¾ pound calcium cyanamid + 4 pounds acid phosphate.	975	38	....	....
14	.....do.............	A, 1 pound calcium cyanamid + 6 pounds acid phosphate.	1,025	35	1,247	1,574
		B, 1 pound calcium cyanamid + 6 pounds acid phosphate.	1,263	20	1,901	
15	.....do.............	A, 1½ pounds calcium cyanamid + 2½ pounds acid phosphate.	62	62	263	163
		B, 1½ pounds calcium cyanamid + 2½ pounds acid phosphate.	61	62	62	
16	Calcium cyanamid in solution.	A, 4 per cent solution of calcium cyanamid, 5 gallons to 4 bushels manure.	598	34	....	....
		B, 4 per cent solution of calcium cyanamid, 5 gallons to 4 bushels manure.	327	64	....	....
		C, 3 per cent solution of calcium cyanamid, 5 gallons to 4 bushels manure.	677	25.2	1,173	904
		D, 3 per cent solution of calcium cyanamid, 5 gallons to 4 bushels manure.	312	65.5	635	
17	.....do.............	A, 4 per cent solution of calcium cyanamid, 2½ gallons to 2 bushels manure.	263	17	552	314
		B, 4 per cent solution of calcium cyanamid, 2½ gallons to 2 bushels manure.	11	96.5	81	

When calcium cyanamid was present in mixtures at the rate of one-half pound per bushel of manure, it is apparent from the results given in Table 6, No. 4, that a satisfactory larvicidal action was obtained. It is evident that practically four times as much cyanamid is required in mixtures for the effective treatment of open piles as for cages or boxes.

CALCIUM CYANAMID, MURIATE OF POTASH (KCl), AND ACID PHOSPHATE.

This mixture, though similar to the one just discussed, showed no apparent larvicidal action in the amounts employed (Table 4, No. 4).

TABLE 5.—*Destruction of fly larvæ in horse manure—Results using calcium cyanamid and acid phosphate mixture.*

[Cage experiments at Bethesda, Md., and box experiments at Baton Rouge, La., 1915.]

No.	Treatment of 8 bushels manure followed by sprinkling with 10 gallons of water.	Flies emerged.	Apparent larvicidal action (per cent of control average).	Number bacteria per gram calculated to dry weight.	Manure, total nitrogen.	Water extract.			
						Alkalinity, cc N/20 NaOH per 100 cc (5 grams manure).	In per cent of total nitrogen.		
							Nitrogen.	Ammonia nitrogen.	Amid nitrogen.
		Number.		*Millions.*	*Per ct.*		*Per ct.*	*Per ct.*	*Per ct.*
1	A, 1 pound calcium cyanamid +1½ pounds acid phosphate, cages	7	98.7	3,608	0.533	6.25	33.60	2.40	10.73
	B, Do	1	99.0	2,646	.456	5.75	36.84	4.20	11.05
	C, Control	982	....	18,750	.449	7.25	29.84	2.90	9.57
	D, Do	35	....	25,500	.379	8.00	29.53	3.60	11.17
2	A, 1 pound calcium cyanamid +8 pounds acid phosphate, cages	472	83.8	20.5	.519	3.25	25.82	5.97	....
	B, Do	317	88.6	10	.519	3.00	28.71	9.06	....
	C, Control	4,410	....	36	.440	3.75	25.45	1.70	....
	D, Do	2,665	....	50	.582	4.00	21.14	1.72	....
3	A,[1] 1 pound calcium cyanamid+6 pounds acid phosphate, boxes	135	95.9	1,600	.442	1.50	38.01	11.76	13.58
	B, 1⅓ pounds calcium cyanamid+6 pounds acid phosphate, boxes	650	80.0	900	.428	4.00	34.11	11.91	14.26
	C,[1] 2 pounds calcium cyanamid+12 pounds acid phosphate, boxes	307	90.5	800	.449	3.50	24.94	11.80	25.62
	D,[1] Control	3,238	....	1,400	.379	2.75	29.55	7.92	13.19

[1] 3 A, C, and D contained nitrates.

TABLE 6.—*Destruction of fly larvæ in horse manure—Results using 4 per cent solutions of calcium cyanamid (series 1 and 2), and with dry calcium cyanamid and acid phosphate mixtures (series 3 and 4).*

[Open pile experiments, Baton Rouge, La., 1915.]

No.	Treatment of 8 bushels manure.	Flies emerged.	Apparent larvicidal action (per cent of control average).	Number bacteria per gram manure calculated to dry weight.	Manure, total nitrogen.	Water extract.			
						Alkalinity, cc N/20 NaOH per 100 cc (5 grams manure).	In per cent of total nitrogen.		
							Nitrogen.	Ammonia nitrogen.	Amid nitrogen.
		Number.		*Millions.*	*Per ct.*		*Per ct.*	*Per ct.*	*Per ct.*
1	A, 10 gallons 4 per cent calcium cyanamid solution	(1)	100	2,222	0.735	7.35	45.03	6.26	24.35
	B, Do	(1)	100	600	.604	6.85	35.26	7.28	18.22
	C, Control	(1)	....	347	.653	7.00	30.93	6.74	12.56
	D, Do	(1)	....	857	.688	7.50	34.30	5.96	8.28
[2] 2	A, 10 gallons 4 per cent calcium cyanamid solution	924	90.5	1,458	.428	6.75	36.68	11.68	11.50
	B, Do	1,865	79.9	1,154	.435	2.50	27.59	9.66	6.43
	C, Control	9,589	....	909	.449	2.75	21.16	8.69	6.90
	D, Do	8,969	....	723	.423	3.75	29.31	8.97	4.11
[2] 3	A, 3 pounds calcium cyanamid+12 pounds acid phosphate, 10 gallons water added	70	57	5,745	.744	3.75	27.98	8.07	10.73
	B, Do	16	90	2,000	.646	3.50	24.30	7.89	13.78
	C, Control	263	....	4,285	.604	3.00	20.53	5.79	3.48
	D, Do	62	....	4,151	.540	1.75	23.33	7.04	3.33
[2] 4	A, 4 pounds calcium cyanamid+4 pounds acid phosphate, 10 gallons water added	19	97	182	.625	3.75	26.88	5.76	22.16
	B, Do	4	99	545	.510	5.50	35.30	6.67	20.78
	C, 5 pounds calcium cyanamid+5 pounds acid phosphate [3]	5	99	255	.646	4.25	34.68	6.81	21.37
	D, Do	15	98	1,000	.510	4.60	46.27	9.02	23.92
	G, 3 pounds calcium cyanamid+6 pounds kainit [3]	4	99	1,220	.681	6.25	36.27	6.90	19.83
	H, Do	13	98	511	.744	6.85	45.29	8.47	17.87
	E, Control [3]	511	....	431	.414	3.10	27.05	7.24	16.43
	F, Do	727	....	511	.470	3.35	27.44	7.02	13.83

[1] Piles examined for larvæ on third day after treatment.
[2] Nitrates were present in all samples of series 2, 3, and 4.
[3] 10 gallons of water added per 8 bushels of manure.

CALCIUM CYANAMID AND KAINIT.

The results given in Table 4, No. 1, where 1 pound of each ingredient was used in duplicate tests, show varying and inconsistent action, 50 and 81 per cent. Open-pile experiments given in Table 6, No. 4, G and H, show that where 3 pounds of cyanamid and 6 pounds of kainit were applied in mixture practically all the larvæ were killed.

Chemical and bacteriological examinations were made of samples of manure taken from the last two mentioned experiments. The bacterial count in one sample was twice as high as in the untreated control samples and the other sample gave the same result as the controls. The percentage of total nitrogen, as well as of the various nitrogenous constituents, in the two treated samples, was markedly higher than in the two controls, and the alkalinity also was increased. These changes were due to the presence of nitrogen and lime in the calcium cyanamid.

CALCIUM CYANAMID AND ACID PHOSPHATE.

Varying amounts of calcium cyanamid and acid phosphate were used in preparing mixtures in order to determine the most effective combination. The results of 5 cage experiments at Bethesda, Md., are recorded (Table 3, Nos. 1 and 2). From three-fourths pound to 2 pounds of cyanamid and from 2 to 6 pounds of acid phosphate were used in the various mixtures tested. The larvicidal results, from 90 to 99 per cent, with mixtures containing 1 pound or more of the cyanamid were very satisfactory. It is evident from the results in the table that three-fourths of a pound of cyanamid is not sufficient. Some additional cage experiments and one series of tests in boxes are recorded in Table 5. In the cage experiments mixtures of 1 pound of cyanamid with 1½ pounds of acid phosphate, and 1 pound of cyanamid with 8 pounds of acid phosphate were tested in duplicate. The results agree with those in Table 3, showing that 1 pound of cyanamid in cage experiments was a fairly effective application.

Bacteriological and chemical analyses of samples of manure from the cages are given in Table 5, Nos. 1 and 2. The bacteria were reduced in both series by the treatment, alkalinity was slightly reduced, and the water-soluble and ammonia nitrogen results showed a tendency to increase. The ammonia results in No. 2, where the 8 pounds of acid phosphate were used, were markedly higher than the controls.

The results of the experiments in boxes are given in Table 5, No. 3. They show an apparent larvicidal action averaging 89 per cent. It is probable that the surface exposure of the manure in the boxes is the important feature in determining the effectiveness of this treatment, although these three experiments do not bring this out as fully as might be expected. Box A had a surface exposure of 5 square feet, box B of 8 square feet, box C of 7 square feet, and box D of 7½ square feet.

The bacteria counts averaged 75 per cent as high as for the control sample, 3D. The alkalinity of the water extracts of the manure showed no definite change in any case. The water-soluble nitrogen was increased in two samples, and reduced in the third; the ammonia nitrogen was decidedly increased in the treated samples, while the amid nitrogen was increased only in sample 3C.

The results of several open-pile experiments are shown in Table 4, Nos. 5 to 15, inclusive. The mixtures employed contained from one-third of a pound to 1½ pounds of calcium cyanamid, and from three-fourths of a pound to 6 pounds of acid phosphate per 4 bushels of manure. The larvicidal results were very irregular, but it is evident that 1 pound of cyanamid is not effective in open piles.

Some results of the effect of larger applications of mixtures of cyanamid and acid phosphate in open-pile experiments at Baton Rouge are recorded in Table 6, Nos. 3 and 4.

In series No. 3 the mixture contained 3 pounds of cyanamid and 12 pounds of acid phosphate per 8 bushels of manure. From the two control piles 263 and 62 flies emerged, but using the average of these two figures, a 57 per cent and a 90 per cent larvicidal action was apparently obtained. Attention is called to the fact that from cage 3A, 70 flies emerged, a count higher than the lower control. On account of the uneven infestation of the manure used in these tests the percentages have little value. The number of bacteria in 3B, where a 90 per cent larvicidal action was obtained, was reduced 50 per cent by the treatment. The total nitrogen of the manure apparently was increased by the treatment, as was the water-soluble and ammonia nitrogen. The greatest increase was in the amid nitrogen. There was apparently a slight increase in the alkalinity of the water extracts. Nitrates were detected in all of these samples.

In No. 4, Table 6, 4 and 5 pounds of cyanamid were mixed with equal amounts of acid phosphate. With both mixtures the larvicidal results were highly satisfactory, 97 and 99 per cent of the larvæ being destroyed. No reduction in the number of bacteria in the treated samples was found. An increased percentage of total and amid nitrogen due to the nitrogen of the cyanamid was obtained in all of the 4 samples, while 3 of the 4 samples showed an increase in the water-soluble nitrogen. Less ammonia nitrogen was found in 3 of the 4 samples than in the controls. The alkalinity slightly increased in all cases. All the samples showed the presence of nitrates by the diphenylamin method, the color reactions being stronger in the treated than in the untreated samples.

Many temperature readings of the manure treated with mixtures of cyanamid and acid phosphate were taken. In practically all instances the results of the readings in the case of the treated samples were the same as in the case of the controls. This is in line with the bacterial counts and indicates that no marked action on the bacterial flora had taken place.

It is probable that the cyanamid is the principal toxic constituent of the various mixtures, and the cyanamid which is soluble in water is undoubtedly more toxic than its polymer dicyandiamid.

Kionka (5) has reported experiments showing that 0.1 gram of pure cyanamid is a fatal dose for dogs, when given subcutaneously, while it takes 0.25 gram of dicyandiamid to produce the fatal result. The fatal doses for dogs when administered internally are, respectively, 0.75 and 2 grams. These experiments indicate that cyanamid is three times as toxic as dicyandiamid.

SUSPENSIONS OF CALCIUM CYANAMID.

As the substances which have been found to be effective larvicides act most effectively when applied in solution or suspension, it was considered advisable to test the effect of 3 and 4 per cent solutions of calcium cyanamid. The results of these experiments are recorded in Table 4, Nos. 16 and 17, and Table 6, Nos. 1 and 2.

The results recorded in Table 4, Nos. 16 and 17, can hardly be compared with the other open-pile experiments for the reason that only 2 bushels of manure instead of 4 were used at each treatment. In No. 16 the application was made twice; that is, there was a total of 4 bushels of manure in the pile instead of 12 as in other experiments. In No. 17 only one application was made to each of the 2-bushel piles. Moreover, only large larvæ, probably 3 days old, were present in the manure at the time of treatment. It is not clear how much these conditions would influence the effectiveness of the solution used, but at any rate the larvicidal results were all low and irregular, except in one experiment, No. 17–B.

In series 2, Table 6, where two treatments were applied to each of 4 bushels of manure, in duplicate, one result was rather low (80 per cent), while one was fairly good (90 per cent). In series 1, Table 6, cages were not placed over these piles to catch emerging flies, but an estimate of the larvicidal effect was made from examination of the piles on the third day after treatment. At that time no larvæ were seen in 1A and only one or two in 1B, while the control piles were seen to be heavily infested. In general, the results with solutions of cyanamid were not uniformly favorable, as far as larvicidal action is concerned, and it would require a considerable number of additional experiments to determine just what strength would be uniformly effective.

The bacteria of series 1 and 2, Table 6, showed an increase in three of the four treated samples. No increase of total nitrogen was found, and but one sample, 2A, showed an increase in alkalinity. The water-soluble nitrogen, ammonia nitrogen, and amid nitrogen in the water extracts of the treated samples of both series showed a tendency to increase. Nitrates were detected in all samples of series 2, but were absent from all samples in series 1. In the former series the manure was older, the piles having stood longer before the samples were taken than with series 1.

COMMERCIAL MIXED FERTILIZERS CONTAINING CALCIUM CYANAMID.

Calcium cyanamid is frequently used to furnish the nitrogen of mixed fertilizers, either in whole or in part. Such fertilizers usually do not contain any calcium cyanamid as such, since the cyanamid has been decomposed into urea, ammonia, etc. The percentage of calcium cyanamid used in commercial mixed fertilizers varies with each brand. The commercial fertilizer which was employed in this test contained 12¼ per cent phosphoric acid, 2¾ per cent ammonia, and 2¼ per cent potash.

One-third of a bushel of manure was treated with 1 pound of the mixed fertilizer in a box covered with netting. The sample was then well moistened. Only 2 flies emerged from the box which was treated with the mixed fertilizer, while 909 flies emerged from the control box. The application of the mixed fertilizer in this experiment was a very heavy one, and as but one experiment was carried out, and that one on a small scale, no conclusion can be drawn. As manufacturers employ varying amounts of cyanamid in their mixtures it is impossible to use commercial mixtures as a larvicide with any assurance of obtaining satisfactory results without first testing the mixture in question and determining the amount necessary to apply to the manure to destroy the maggots.

THE AMOUNTS OF FERTILIZER MIXTURES TO APPLY TO PITS AND PILES OF MANURE TO KILL FLY LARVÆ.

From all of the cage tests and the box experiments (Table 5, No. 3) the indications are that mixtures containing 1 pound of calcium cyanamid and 2 to 4 pounds of acid phosphate will destroy a high percentage of the maggots of the house fly present in 8 bushels of manure, when the manure is placed in such receptacles. In these cases the surface treated was much smaller than in an open pile containing the same amount of manure, and as the surface was practically level the mixture could be applied effectively and forced into the manure by sprinkling with water. It seems likely that the mixtures will act effectively when applied to manure in pits where approximately the same conditions exist as in the box or cage tests; that is, where there is only one surface to be treated. One pound of cyanamid in mixtures was used successfully in a box having a surface exposure of 5 square feet and in cages with a surface of 4 square feet. It is therefore suggested that mixtures containing 1 pound of cyanamid and 2 to 4 pounds of acid phosphate be applied to boxes or pits at the above rate per 4 square feet of surface exposure. The ideal way of storing manure is in a water-tight pit. If this is not available, the manure may be thrown into a large box and then effectively treated with the cyanamid mixture at the above rate.

In the treatment of piles of manure it is evident from the results in Table 6, series 4, that 4 pounds of calcium cyanamid applied in mixtures with either acid phosphate or kainit per 8 bushels of manure acts as an effective larvicide. To be sure of retaining all of the nitrogen and ammonia which ordinarily is lost from a pile of manure, a higher ratio of acid phosphate to calcium cyanamid than was used in these tests should be employed. The chemical analyses of No. 4, Table 6, show no changes from the control samples, but the results of Table 5, No. 2, show the advantage of using a large proportion of acid phosphate.

Kainit may be employed in the mixtures together with the cyanamid and acid phosphate. Thus potash, as well as lime, nitrogen, and phosphoric acid, will be added to the manure. The substitution of kainit for part of the acid phosphate makes no apparent difference in the larvicidal action of the mixture. It is important to apply in the mixture one-half pound of calcium cyanamid per bushel of manure. The manure should be thoroughly sprinkled with water after each application of the cyanamid mixture. It is probable that gypsum may be substituted in whole or in part for the acid phosphate.

THE VALUE OF USING CALCIUM CYANAMID, ACID PHOSPHATE, AND KAINIT ON MANURE.

It is likely that calcium cyanamid, which contains lime in amounts equivalent to 70 parts of $Ca(OH)_2$ per 100, if used alone on fresh manure in any considerable amount, will expel some of the nitrogen and ammonia present in the manure. Acid phosphate was used in the mixtures with the cyanamid, in part to neutralize the lime, and also to hold the nitrogen and ammonia which might be expelled by the lime of the cyanamid, as well as to retain the nitrogen and ammonia lost during ordinary fermentation processes. During the decomposition of calcium cyanamid in the soil the lime is gradually precipitated as carbonate, and this same process undoubtedly takes place in the manure. Acid phosphate, kainit, and gypsum are quite extensively recommended for the purpose of preserving manure. Commercial acid phosphate contains about 14 per cent of available phosphoric acid; kainit contains approximately 12.5 per cent of potash, and cyanamid contains 20 to 22 per cent of ammonia. It is accordingly evident that when the above mixture is applied to manure the three essential fertilizing elements are added in considerable amounts, and if acid phosphate is used in a ratio of 6 or 8 to 1 no loss of ammonia will result, but the nitrogen and ammonia which escape during the normal fermentation of manure will be retained.

THE COST OF EFFECTIVE FERTILIZER MIXTURES.

Under normal conditions kainit sells for $12 to $14 per ton, making the cost about two-thirds of a cent a pound. Kainit is not available at the present time. Calcium cyanamid was quoted January 1, 1916, at $2.45 per unit of ammonia. Calculating the average percentage of ammonia present in cyanamid at 21 per cent, the cost per ton is $51.45, or 2.6 cents per pound in ton lots. Cyanamid is at present handled only in large amounts and by manufacturers of mixed fertilizers. It requires only the demand to make it available in all parts of the country. During the fall of 1915, several lots of acid phosphate were purchased, and the average price, when purchased in hundred-pound lots, was 1 cent per pound. It was quoted at $14 per ton January 1, 1916. Four pounds of the calcium cyanamid and 4 pounds of acid phosphate, as seen in Table 6, series 4, showed an effective larvicidal action. The cost of this mixture was 14½ cents per 8 bushels of manure, or 1.8 cents per bushel. In Table 6, No. 4, the results also show that a mixture composed of 3 pounds of calcium cyanamid and 6 pounds of kainit gave an effective larvicidal action. The cost of the treatment in this case was 1½ cents per bushel. In the experiments in cages and in boxes mixtures containing 1 pound of the cyanamid and 2 pounds of acid phosphate per 8 bushels have given satisfactory results. The cost in this case was one-half cent per bushel.

From these figures it appears that the fertilizer mixtures applied in sufficient amounts to act as effective larvicides on open piles are more expensive than borax or powdered hellebore. However, in the former treatment the fertilizing value of the manure is greatly increased, and when considered from this point of view the actual larvicidal cost of the fertilizer mixtures is materially lessened.

THE AMOUNTS OF MANURE TREATED WITH FERTILIZER MIXTURES TO APPLY TO THE SOIL.

One hundred and fifty pounds of calcium cyanamid per acre is stated by Pranke (6) to be the most economical amount to apply to the soil at one time. When manure is treated in boxes or pits at the rate of 1 pound of calcium cyanamid and 2 pounds of acid phosphate per 8 bushels (10 cubic feet) of manure, taking the weight of 8 bushels of fresh manure at 120 pounds, it is calculated that 16⅔ pounds of calcium cyanamid and 33 pounds of acid phosphate will be present per ton of fresh manure. If it is desired, kainit may be used in place of a part of the acid phosphate in the mixture. Manure treated in this manner and applied to the soil at the rate of 9 tons per acre will furnish 150 pounds of calcium cyanamid, which is mentioned above as being the most economical application of the cyanamid. The application of more than 9 tons per acre would be uneconomical.

When piles of manure are treated with a mixture containing 4 pounds of calcium cyanamid and 4 pounds of acid phosphate, these two substances are added to the manure at the rate of 65 pounds per ton. As above, the weight of fresh manure is taken at 15 pounds per bushel. The manure treated with this mixture should be applied to the soil at the rate of only 2½ tons per acre.

In connection with the use of cyanamid as a fertilizer, the following statements of E. J. Pranke (6) are of interest:

> Since cyanamid is a medium slow-acting fertilizer, it should be applied to the crop not less than 70 to 80 days before the harvest, in order that the nitrogen may be completely utilized by that crop. Experience has shown that the most economical utilization of a nitrogen fertilizer is in conjunction with the other fertilizer elements, phosphorus and potash. For this reason it is recommended that cyanamid be used as a part of a fertilizer mixture rather than it be applied alone.
>
> If cyanamid is to be applied to very acid soils, such soils should be put in a productive condition by judicious liming sometime before the application of the fertilizer. The application of barnyard manure will help to establish the bacteria that are deficient in such soils.

EXPERIMENTS WITH BORIC ACID AND SULPHUR.

BORIC ACID, $B(OH)_3$.

Two open-pile experiments were carried out, using solutions of boric acid, one-fourth pound to 5 gallons of water, and applying the same to 4 bushels of manure. The tests showed a 97.4 per cent and a 99.8 per cent larvicidal action. Boric acid was quoted wholesale January 1, 1916, at 10 to 10½ cents per pound for commercial grade. Based on the price just quoted, the cost of treating manure with boric acid, judging from these two tests, was five-eighths cent per bushel of manure. It is probable that somewhat smaller amounts of boric acid than one-half pound per 10 gallons of water may be effectively employed. Borax cost, January 1, 1916, from 5¾ to 6 cents per pound wholesale, or 4 cents for two-thirds of a pound, a quantity sufficient to destroy all the maggots in 8 bushels of manure. The cost was therefore one-half cent per bushel of manure. It is apparent that boric acid is equally effective as borax as a larvicide, but it has not been determined whether there is any difference in the action of these two substances on plant growth, although from the few tests reported in the literature no differences are indicated.

SULPHUR.

One box test in which one-third of a bushel of manure and 5⅓ ounces of flowers of sulphur were used was carried out at the suggestion of Prof. C. V. Piper. A control was employed, both samples being moistened and a large number of fly eggs added to each. Forty-two flies emerged from the box treated with sulphur and 909 flies from the control. The application of 1 pound of sulphur per bushel of manure is a large one, and, if used on a large scale, would not only involve considerable expense, but would undoubtedly have an injurious action on plants when applied to the soil.

SUMMARY.

During the fly season of 1915, the larvicidal action of infusions of 18 plant materials was tested. None of these, with the exception of hellebore, were sufficiently economical and effective to be classed as practical larvicides; Berberis, Cinchona, and larkspur in the amounts employed showed some larvicidal action.

Various mixtures of calcium cyanamid, acid phosphate, kainit, and gypsum were tested.

One pound of calcium cyanamid in mixtures with 2 to 4 pounds of acid phosphate proved effective in cages and boxes where the surface exposure of the manure was approximately 4 square feet.

A similar mixture may prove effective in pits. In both cases the treatment should be based on the number of square feet of exposed surface of the manure. The cost of this treatment, based on wholesale prices January 1, 1916, is approximately 1.4 cents per square foot.

In several open-pile experiments calcium cyanamid applied in mixtures with acid phosphate or kainit at the rate of one-half pound per bushel of manure showed an apparent larvicidal action of 98 per cent. By using a mixture composed of calcium cyanamid, acid phosphate, and kainit, the three essential plant elements, nitrogen, potash, and phosphoric acid, are added to the manure. The nitrogen and ammonia which usually escape from untreated manure piles will be retained if sufficient acid phosphate is used, and the fly maggots will be killed at the same time. The cost of this treatment, in which one-half pound of calcium cyanamid and one-half pound of acid phosphate are applied for each bushel of manure, is 1.8 cents per bushel.

SUMMARY OF THREE SEASONS' WORK.

From the three seasons' work it appears that borax used at the rate of two-thirds of a pound per 10 gallons of water and sprinkled over 8 bushels of manure is the least expensive and the most effective larvicide. Caution should be used in treating manure to be employed for agricultural purposes, because of the injurious action on plant growth of excessive applications of borax. It is, however, recommended for the treatment of all manure not to be used for agricultural purposes, for refuse piles of all kinds, stable floors, etc. The cost of the borax treatment, when borax sells for 8 cents per pound, is one-half cent per bushel.

Powdered hellebore, using one-half pound per 10 gallons of water, and applying the same to 8 bushels of manure, is an effective larvicide and without action on plant growth. The cost[1] is variable. On

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[1] It is unusually difficult to give a fair retail price for these substances because of the advance in price of chemicals and fertilizers. Borax was quoted retail Washington, D. C. at 8½ to 12 cents per pound in 100-pound lots; powdered hellebore, in 100-pound lots, at 35 cents per pound; acid phosphate $1.75 to $2 for 167-pound sacks. Kainit is not obtainable at the present time; the usual price is $12 per ton, or three-fourths to 1 cent per pound in 100-pound lots. Calcium cyanamid is manufactured by but one company in North America, although there are several companies producing it abroad. This American company does not ordinarily sell calcium cyanamid in less than carload lots. The price was $51.45 per ton f. o. b. Niagara Falls, Ontario. The figures just given were those quoted on March 1, 1916. Calcium cyanamid is handled by manufacturers of mixed fertilizers.

January 1, 1916, the wholesale price was 16 to 17 cents per pound, but it should be obtained in large amounts under ordinary conditions at 11 to 12 cents per pound. The cost of treating manure with solutions of powdered hellebore, when it sells for 17 cents per pound, is therefore 1 cent per bushel.

Solutions of aniline and emulsions of nitrobenzene with fish-oil soap, reported in Department Bulletin 245 (2), also proved to be effective larvicides, and did not injure the manure, as far as could be determined by chemical and bacteriological examination.

The indications are that calcium cyanamid, acid phosphate, and kainit mixtures can be used as effective larvicides, if one-half pound of calcium cyanamid is present in the mixture per bushel of the manure treated. The cost of applying a mixture of one-half pound calcium cyanamid and one-half pound acid phosphate per bushel of manure is 1.8 cents per bushel, but this is materially lessened by the increased fertilizing value of the treated manure.

Among the other substances which have been found effective in reasonable amounts, but can not be recommended because of their extremely toxic action, are potassium cyanid, Paris green, arsenic dip, and pyridine.

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